skyla

Avian & Reptile Panel

PN：900-190

For Veterinary In Vitro Diagnostic Use Only

Rev：D

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1. Intended Use

The skyla Avian & Reptile Panel used with skyla Analyzer, is intended to be used for the quantitative determination of Albumin (ALB), Aspartate Aminotransferase (AST), Total Protein (TP), Glucose (GLU), Calcium (Ca), Chloride (Cl), Phosphorus (PHOS), Potassium (K), Sodium (Na), Bile Acid (BA), Uric Acid (UA) and Creatine Phosphokinase (CPK), and in avian & reptile whole blood, plasma, or serum. The calculated values of Globulin (GLOB), Albumin/Globulin Ratio (A/G Ratio), Sodium/Potassium Ratio (Na/K Ratio) and Corrected Calcium(C-Ca) can then be obtained.

2. Principles

The skyla Avian & Reptile Panel contains a total of 12 types of dried reagent located in the respective detection wells of the reagent disc. The user only needs to inject the blood specimens into the sample port of the disc, and then places the disc into the analyzer. The test will be done automatically within 15 minutes. For the detail description of disc, please refer to “skyla Analyzer Operator’s Manual”.

Clinical Significance:

Albumin (ALB): ALB is one of the indicators for kidney function, liver function and dehydration.

Aspartate Aminotransferase (AST): AST is a marker to examine hepatobiliary diseases and the degree of myocardium injury.

Calcium (Ca): Ca can be used to detect parathyroid-related、bone diseases, chronic kidney diseases and tetany of vitamin D deficiency.

Chloride (Cl): Cl is one of indicators for fluid and electrolyte balance. It can be used to evaluate the disorders of vomiting, diarrhea, dehydration and renal failure.

Creatine Phosphokinase (CPK): CPK can be used for the diagnosis of muscle damage, convulsions,

heart disease; hypothyroidism; severe exercise, physical inactivity, decreased muscle mass.

Glucose (GLU): GLU can be used for the diagnosis of diabetes and diseases related to the carbohydrate metabolism.

Phosphate (PHOS): PHOS is an indicator for kidney diseases, hypothyroidism, and malnutrition.

Sodium (Na): Na is one of indicators for fluid and electrolyte balance. It can be used to evaluate the disorders of vomiting, diarrhea, dehydration and Addison's disease.

Potassium (K): K is one of indicators for fluid and electrolyte balance. It can be used to evaluate the disorders of vomiting, diarrhea, dehydration and Addison's disease.

Total Protein (TP): TP is an indicator for function of liver synthesis and the degree of protein-losing caused by kidney diseases.

Bile Acid (BA): BA can be used for the diagnosis of liver disease.

Uric Acid (UA): UA can be used for diagnosis and prognosis tracking of kidney related diseases and diseases caused by metabolic disorders.

Globulin (GLOB): GLOB is calculated from TP and ALB and it is used to assess liver function.

Albumin/Globulin Ratio (A/G Ratio): The A/G Ratio is the ALB and GLOB ratio. It is used to assess liver function.

Sodium / Potassium Ratio (Na/K Ratio): Na/K Ratio may indicate the kidney stress, hyper-aldosteronism and Addison's disease.

Corrected Calcium (C-Ca): C-Ca is calculated from Ca and ALB and it is used to assess Hypocalcaemia

Method:

ALB

ALB is determined through the endpoint chemical reaction method. When ALB is binding to Bromocresol Green (BCG), it forms a yellow-green complex. The absorbance at a wavelength of 600 nm can be measured. The amount of ALB in the sample is proportional to the bound ALB.

AST

AST activity is enzymatically determined. When the test sample reacts with the substrate-enzyme reagent, AST converts L-Aspartic Acid and α-Ketoglutarate into Monosodium Glutamate and Amide Acetate. Amide Acetate is subsequently converted into Malate by Malate Dehydrogenase while NADH undergoes oxidation to NAD. The decrease of NADH absorbance is measured at a

wavelength of 340 nm and is proportional to AST activity.

Ca

Ca is determined through the endpoint chemical reaction approach. Calcium reacts with Arsenazo III and form a purple-colored complex. The complex formation is measured at a wavelength of 650 nm and is proportional to the amount of Ca in the sample.

Cl

Cl is enzymatically determined. Chloride will bind to Amylase and consequently lead to reactivation of the enzyme. Amylase will then convert a synthetic substrate α-(2-Chloro-4-Nitrophenyl)-β-1,4- Galactopyranosylmaltoside (Gal-G2-α-CNP) to 2-chloro-4-nitrophenol (CNP). Its formation and absorption at a wavelength of 405 nm is proportional to the amount of Chloride in the sample.

CPK

CPK is enzymatically determined. CPK catalyzes the Creatine Phosphate and ADP to form a Creatine and ATP. Then Hexokinase catalyzed the Glucose and ATP, produces the D-Glucose-6-Phosphate (G-6-P). In the presence of NAD, G-6-PD converts G-6-P into 6-Phosphogluconate and NADH. The absorbance at the wavelength of 340nm can be measured in the presence of NADH. The absorbance is proportional to the CPK concentration.

GLU

GLU is determined through the endpoint enzymatic reaction approach. The Sucrose is catalyzed by Hexokinase to D-Glucose-6-Phosphate (G-6-P). In the presence of NAD, G-6-PD converts G-6-P into 6- Phosphogluconate and NADH. The absorbance at a wavelength of 340 nm can be measured in the presence of NADH. The absorbance is proportional to the GLU concentration.

PHOS

PHOS is enzymatically determined. By going through a series of enzymatic reactions with Sucrose Phosphorylase, Phosphoglucomutase, and Glucose-6-Phosphate Dehydrogenase, PHOS forms 6-Phosphogluconate and NADH. And NADH is measured at a wavelength of 340 nm and is proportional to the amount of PHOS in the sample.

K

K is enzymatically determined. Pyruvate Kinase (PK) dephosphorylates Phosphoenolpyruvate (PEP) to form Pyruvate. Then the Pyruvate converts to Lactate under catalysis of Lactate Dehydrogenase (LDH). At the same time, NADH is oxidized to NAD+ which in turn undergoes a color reaction. The rate of change of absorbance at wavelength of 340 nm is measured and proportional to the potassium in the sample.

Na

Na is enzymatically determined. By going through the activation of β-Galactosidase with Na ion, o-Nitrophenyl-β-Galactopyranoside (ONPG) is further catalyzed by activated β-Galactosidase,

form o-Nitrophenol and Galactose. The absorbance caused by o-Nitrophenol is measured at a wavelength of 405 nm and is proportional to the amount of Na in the sample.

TP

TP is determined by the Biuret method. The peptide bonds of the protein react with copper ions in an alkaline environment and form a purple compound. The color development is proportional to the original TP concentration and is measured at a wavelength of 546 nm.

BA

BA is enzymatically determined. In the presence of Thio-NAD^+, Bile acids reacts with enzyme 3-α-hydroxysteroid dehydrogenase (3-α-HSD) to form Oxidized bile acids and Thio-NADH. The enzyme cycling occurs, when NADH is present in the reaction, 3-α-HSD convert Oxidized bile acids back to Bile acids. The formation rate of Thio-NADH is proportional to the BA concentration in the sample. BA concentration is quantitated by measuring the absorbance at wavelength of 405 nm.

UA

UA is determined enzymatically by an endpoint reaction. In this method, Uric Acid is converted into Allantoin and Peroxide. The Peroxidase catalyzed reaction of Peroxide with 4-Aminoantipyrine (4-AAP) and 3,5-Dichloro+2-Hydroxybenzene-Sulfonate (DCHBS) results in the formation of a Quinoneimine dye. The dye formation is proportional to the UA concentration and is measured at wavelength of 510 nm.

Reaction pathway:

ALB

$$\text{Albumin} + \text{BCG} \longrightarrow \text{Albumin-BCG Complex}$$

AST

$$\text{L-Asparate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloactate} + \text{L-Glutamate}$$

$$\text{Oxaloactate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + NAD^+$$

Ca

$$Ca^{2+} + \text{ArsenazoIII} \longrightarrow Ca^{2+}\text{- ArsenazoIII Complex}$$

Cl

$$\text{EDTA-}Ca^{2+} + \alpha\text{-Amylase} \xrightarrow{Cl^-} \text{EDTA} + \alpha\text{-Amylase-}Ca^{2+}$$

$$\text{Gal-G2-}\alpha\text{-CNP} \xrightarrow{\alpha\text{-Amylase-}Ca^{2+}} \text{Gal-G2} + \text{CNP}$$

CPK

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\text{CPK}} \text{Creatine} + \text{ATP}$$

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{D-Glucose-6-Phosphate} + \text{ADP}$$

$$\text{D-Glucose-6-Phosphate} + \text{NAD} \xrightarrow{\text{G-6-PDH}} \text{6- Phosphogluconate} + \text{NADH} + \text{H}^+$$

GLU

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{D-Glucose-6-Phosphate} + \text{ADP}$$

$$\text{D-Glucose-6-Phosphate} + \text{NAD} \xrightarrow{\text{G-6-PDH}} \text{6- Phosphogluconate} + \text{NADH} + \text{H}^+$$

PHOS

$$\text{Sucrose} + \text{Pi} \xrightarrow{\text{SP}} \alpha\text{-D-Glucose-1-Phosphate} + \text{D-Fructose}$$

$$\alpha\text{ -D-Glucose-1-Phosphate} \xrightarrow{\text{PGM}} \alpha\text{ -D-Glucose-6-Phosphate}$$

$$\alpha\text{ -D-Glucose-6-Phosphate} + \text{NAD}^+ \xrightarrow{\text{G6PDH}} \text{6-Phospho-D-Gluconate} + \text{NADH} + \text{H}^+$$

K

$$\text{ADP} + \text{PEP} \xrightarrow{\text{K}^+\text{, PK}} \text{Pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

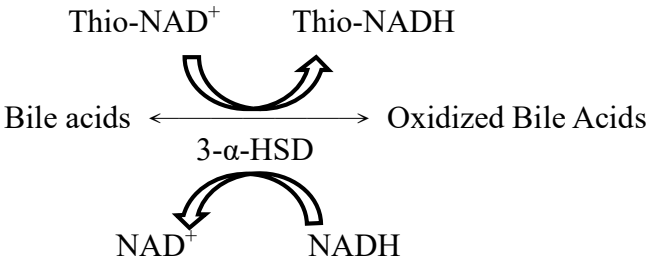
Na

$$\beta\text{-Galactosidase} + \text{ONPG} \xrightarrow{\text{Na}^+} \text{Galactose} + \text{o-Nitrophenol}$$

TP

$$\text{Total protein} + \text{Cu}^{2+} \xrightarrow{\text{Alkali}} \text{Cu-Protein Complex}$$

BA



UA

$$\text{Uric Acid} + O_2 + H_20 \xrightarrow{\text{Uricase}} \text{Allantoin} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{4-AAP} + \text{DCHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H_2O$$

3. Reagents

Included:

Each panel contains dried reagent beads, dried internal QC beads and the diluent.

Reagent Composition:

Composition	Quantity/Panel
4-Aminoantipyrine	0.02 mg
4-Nitrophenyl phosphate disodium salt	0.1 mg
1,4-piperazinediethanesulfonic acid	0.08 mg
Adenosine 5'-monophosphate disodium salt	0.05 mg
ADP	0.05 mg
Arsenazo Ⅲ	0.007 mg
Bromocresol Green sodium salt	5.4 ug
Copper sulphate	0.1 mg
Creatine Phosphate	0.3 mg
D-Glucose	0.1 mg
EDTA calcium disodium salt	0.4 mg
G6PDH	0.35 U
Gal-G2-α-CNP	0.1 mg
Glycylglycine	0.38 mg
Hexokinase	0.2 U
Lactate Dehydrogenase	0.6 U
L-Aspartic Acid	1 mg
LNAC	0.1 mg

Composition	Quantity/Panel
Magnesium Acetate	0.05 mg
Malate Drhydrogenase	0.04 U
NAD	0.08 mg
NADH	0.15 mg
ONPG	0.04 mg
Peroxidase	0.6 U
Phospho(enol)pyruvic acid monosodium salt hydrate	0.02 mg
Phosphoglucomutase	0.05 U
Pyruvate Kinase	0.05 U
Sodium 3,5-dichloro-2-hydroxybenzenesulfonate	0.1 mg
Sucrose	0.3 mg
Sucrose Phosphorylase	0.01 U
Uricase	0.3U
α-Amylase	0.2 U
α-Ketoglutaric Acid	0.05 mg
α-Hydroxysteroid Dehydrogenase	0.02 U
β-Galactosidase	0.3 U
Thio-NAD	0.02 mg
3-α-HSD	0.02 U

Reagent Storage:

- The reagent disc should be stored at 2～8°C.
- The expiry date of the reagent is printed on the outside of the sealed pouch of reagent disc. Do not use if the reagent disc has expired.

4. Specimen Collection and Preparation

Specimen Collection:

- Specimens suitable for skyla Avian & Reptile Panel include lithium heparinized whole blood, lithium heparinized plasma, serum and quality control materials. The sample requirement is 200 μL. (± 10 μL tolerance are allowable)
- If applicable, local regulatory or standard operating procedures of your organization should be followed for the collection, preservation and handling of specimens.

Note: Do not use specimens containing other coagulants. That would cause an incorrect test results.

Specimen Preparation:

- Before applying a sample to the reagent disc, gently rotate the sample tube up and down several times, to confirm the sample is homogeneous (evenly mixed). If the sample is whole blood, do not shake the sample container vigorously to avoid occurrence of hemolysis.

Note:

1. Perform testing within 10 minutes after applying the sample to the reagent disc.
2. The use of whole blood specimens with hematocrits (Hct) higher than 60% may affect the test results.

Note: For further information in specimen collection and preparation, please refer to "skyla Analyzer Operator's Manual"

5. Test Procedures

Material Preparation:

1 piece of the reagent disc of skyla Avian & Reptile Panel

Required materials not included in the panel:

skyla Analyzer

Sample collection container

Micropipette / Tips

Test Conditions:

Test should be carried out in an environment with temperatures of 10°C~32°C. Each test will take about 15 minutes. During the test, chamber in the analyzer keeps the temperature at 37°C for stable assay.

Test Steps:

1. Open the aluminum pouch and remove the reagent disc.
2. Remove the diluent container sealing.
3. Using a micropipette to inject 200 μL of the sample into the reagent disc through the sample port.
4. Press the "start" button on the screen to initiate testing.
5. Place the reagent disc to the analyzer drawer, and press the "ok" button on the screen to analysis.

For details on the operating steps and instrument setting, please refer to “skyla Analyzer Operator’s Manual”.

Note:

1. To operate the reagent disc or instrument, please wear lab gloves and other protective gear to avoid contamination by specimen.
2. The used reagent disc and tips should be discarded as biomedical waste, and treat according to the local legal requirements.
3. Testing should be performed within 20 minutes after the pouch is opened.
4. Do not place the reagent disc at the environment more than 25°C and longer than 48 hours prior to use.
5. If the reagent disc or its package is damaged or is over the expiry date, do not use it.

6. Calibration

The barcode on every manufactured reagent disc contains all information required for calibration of the test items. The analyzer will automatically read the barcode information during testing.

7. Quality Control

- Please refer to the instruction manual for the preparation and use of quality control materials. For discrepancy results, the confirmatory test was suggested to carry out with the automated laboratory analyzer, or to contact with our technical support.
- External quality control materials can be used for the accuracy monitor of skyla system. The recommended frequency of QC testing is as follows, otherwise please follow local legal requirements or the standard operating procedures of your organization
  - At least every 30 days.
  - Before a new batch of reagents is used for testing.
  - When the analyzer was moved or the operating environment significantly changed.

8. Reference interval

It is recommended that every laboratory or test site should establish its own reference interval from its patient population.

9. Limitation

Physiological interferences in blood include hemolysis, icterus, and lipemia. For every test item, 2 Levels serum pool supplemented with known concentrations of the endogenous substances were used for the testing. Significant interference is defined as a >20% shift in the test result. (Note: Highest tested concentration for Hemoglobin: 600 mg/dL; Bilirubin (unconjugated): 62.5 mg/dL, Bilirubin (conjugated): 57.5 mg/dL; Intralipid: 0.55%)

Test Item	Substance concentration with interferences of less than 20%			
	Hemoglobin	Bilirubin (unconjugated)	Bilirubin (conjugated)	Intralipid
ALB	300 mg/dL	62.5 mg/dL	57.5 mg/dL	0.2%
AST	300 mg/dL	42.1 mg/dL	22.3 mg/dL	0.1%
Ca	600 mg/dL	56.3 mg/dL	57.5 mg/dL	0.3%
Cl	300 mg/dL	47.1 mg/dL	44.9 mg/dL	0. 4%
CPK	700 mg/dL	50.9 mg/dL	51.3 mg/dL	0.3 %
GLU	600 mg/dL	62.5 mg/dL	57.5 mg/dL	0.3%
PHOS	500 mg/dL	42.1 mg/dL	57.5 mg/dL	0.13%
K	100 mg/dL	33.5 mg/dL	22.8 mg/dL	0.15%
Na	600 mg/dL	40.2 mg/dL	39.8 mg/dL	0.2%
TP	300 mg/dL	62.5mg/dL	57.5 mg/dL	0.2%
BA	200 mg/dL	50.4 mg/dL	26.6 mg/dL	0.2%
UA	253.1 mg/dL	9.8 mg/dL	6.26 mg/dL	0.03%

10. Performance Characteristics

Dynamic range:

The dynamic range for each test item showed as below.

Test Item	Dynamic Range		Dynamic Range (SI Unit)	
ALB	1.0-6.0	g/dL	10-60	g/L
AST	20-1000	U/L	20-1000	U/L
Ca	4-15	mg/dL	1.0-3.8	mmol/L
Cl	70 - 140	mmol/L	70 - 140	mmol/L
CPK	40-2400	U/L	40-2400	U/L
GLU	30-550	mg/dL	1.7-30.5	mmol/L
PHOS	0.4 - 18.0	mg/dL	0.13 – 5.81	mmol/L
K	1.5-8.5	mmol/L	1.5-8.5	mmol/L
Na	110-175	mmol/L	110-175	mmol/L
TP	1.5-10.0	g/dL	15-100	g/L
BA	5.0-140	μmol/L	5.0-140	μmol/L
UA	1 – 20	mg/dL	59 – 1190	μmol/L

Method Comparison:

The SIMENS ADVIA 1800 was used as comparative method in the study. The tests are performed by using the same clinical serum sample for two methods.

Marker		R^2	Slope	Intercept	Sample No.	Sample Range
ALB	Canine	0.9848	0.9999	0.0000	38	2.7-5.9 g/dL
	Feline	0.9676	1.0000	0.0000	38	3.1-6.4 g/dL
	Equine	0.9597	1.0173	-0.0655	30	3.2-4.3 g/dL
AST	Canine	0.9990	0.9968	0.7497	38	22-803 U/L
	Feline	0.9997	1.0033	-0.9437	38	22-891 U/L
	Equine	0.9990	0.9993	3.4058	16	188-1310 U/L
Ca	Canine	0.9888	1.0000	0.0000	38	7.3-16.4 mg/dL
	Feline	0.9823	0.9966	0.2615	34	6.3-14.1 mg/dL
	Equine	0.9819	1.0551	-0.7172	38	10.2-16.1 mg/dL
Cl	Canine	0.9804	0.9902	1.30159	36	93-136 mmol/L
	Feline	0.9819	0.9802	2.4583	28	90-146 mmol/L
	Equine	--	--	--	--	--
CPK	Canine	0.9960	0.9931	-0.0083	15	88-1027 U/L
	Feline	0.9971	0.9990	-0.0025	12	121-1861 U/L
	Equine	0.9605	1.0126	-0.7476	20	86-237 U/L
GLU	Canine	0.9953	1.0001	0.0089	43	78-558 mg/dL
	Feline	0.9957	0.9956	2.1761	44	93-549 mg/dL
	Equine	0.9959	1.1018	-2.8485	16	73-520 mg/dL
PHOS	Canine	0.9855	1.0469	-0.5006	23	2.3-13.5 mg/dL
	Feline	0.9862	1.0223	-0.2665	22	4.5-12.2 mg/dL
	Equine	--	--	--	--	--
K	Canine	0.9805	0.9728	0.1666	33	3.9-7.7 mmol/L
	Feline	0.981	1.0343	-0.1891	47	2.3-7.2 mmol/L
	Equine	0.9809	0.9745	0.0953	34	1.8-7.0 mmol/L
Na	Canine	0.9854	0.9969	0.7604	40	116-178 mmol/L
	Feline	0.9863	0.9887	1.5809	47	125-175 mmol/L
	Equine	0.9849	1.0181	2.6927	31	111-167 mmol/L
TP	Canine	0.9603	0.9999	0.0000	38	5.2-9.5 g/dL
	Feline	0.9883	0.9999	0.0000	38	6.3-10.3 g/dL
	Equine	0.9639	1.0153	-0.1318	19	6.0-8.3 g/dL
BA	Canine	0.9878	0.9349	0.6227	21	8.8-137 U/L
	Feline	0.9924	0.9848	-0.7697	20	9.1-131 U/L
	Equine	--	--	--	--	--
UA	Canine	--	--	--	--	--
	Feline	--	--	--	--	--
	Equine	--	--	--	--	--

Symbol Index			
	Catalogue number		Consult instruction for use
	Batch code		Use by
	Manufacturer		CE mark
	Temperature limitation		Caution
	Do not reuse		Sufficient for

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